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The fine structure of the spermatheca of *Pardosa lugubris* (Walckenaer, 1802)

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The fine structure of the spermatheca of *Pardosa lugubris* (Walckenaer, 1802). - The present paper is concerned with the structure of the spermatheca of *Pardosa lugubris*. Different types of pores have been found in the cuticle of the spermatheca. In the spermathecal epithelium we observed distinct cell complexes consisting of secretory and auxiliary cells with a cuticular ductule in its centre, which are leading to so-called primary pores. These pores are suggested as a primitive character in all spider species. A single porous plate located on the spermathecal stalk to which glandular cells lead, might be homologous to the dictynoid pores described by Bennett (1992) and to the secondary pores observed in *Amaurobius fenestralis* (Suhm & Alberti 1993). We found secretion of some material into the spermathecal lumen through the primary and secondary pores. The biological function(s) of this secretion remain unclear, but it may play an important role in sperm maturation, nutrition and displacement.

Key-words: *Pardosa lugubris* - female genitalia - spermatheca - glandular epithelium - primary pore - secondary pore.

INTRODUCTION

Detailed observations on the structure of the secondary copulatory organs of spiders are not only of taxonomic, but also of functional and evolutionary importance. Despite of the necessity for comparative studies, there is only a small number of recent investigations that have provided information on the fine structure of female genitalia in spiders (Lopez & Juberthie-Jupeau 1983; Suhm & Alberti 1993; Uhl 1994). Therefore, we studied the fine structure of the spermatheca of *Pardosa lugubris* with its surrounding epithelium by means of light, scanning and transmission electron microscopy.

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MATERIALS AND METHODS

Pardosa lugubris (Walckenaer, 1802) specimens were collected near Heidelberg, Germany. For light (LM) and transmission electron microscopic (TEM) studies, the surrounding tissues were excised and fixed in ice cold 2.5% glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.6) for two hours, rinsed again in buffer and postfixed for 2 hours in 1% osmium ferrocyanide (KARNOVSKY 1971). After washing in 0.1 M cacodylate and 0.05 M maleate buffer (pH 5.2), tissues were stained en bloc with 1% uranyl acetate in maleate buffer for 1 hour. Specimens were dehydrated in a graded series of ethanol and embedded in Spurr's medium (SPURR 1969). Semithin sections were stained with methylene blue-azur II (RICHARDSON 1960) and examined in a Leitz Aristoplan microscope. Ultrathin sections were stained with alkaline lead citrate (REYNOLDS 1963) for 30–60 s and examined in a Zeiss EM 9. For scanning electron microscopic (SEM) studies the female copulatory organ was cleaned with lactic acid for 5–10 minutes before dehydrated in alcohol and sputtercoated with gold in a Technics-Hummer I sputter and examined in a Philips SEM 505. To expand the male palpal organs they were incubated in hot lactic acid.

RESULTS

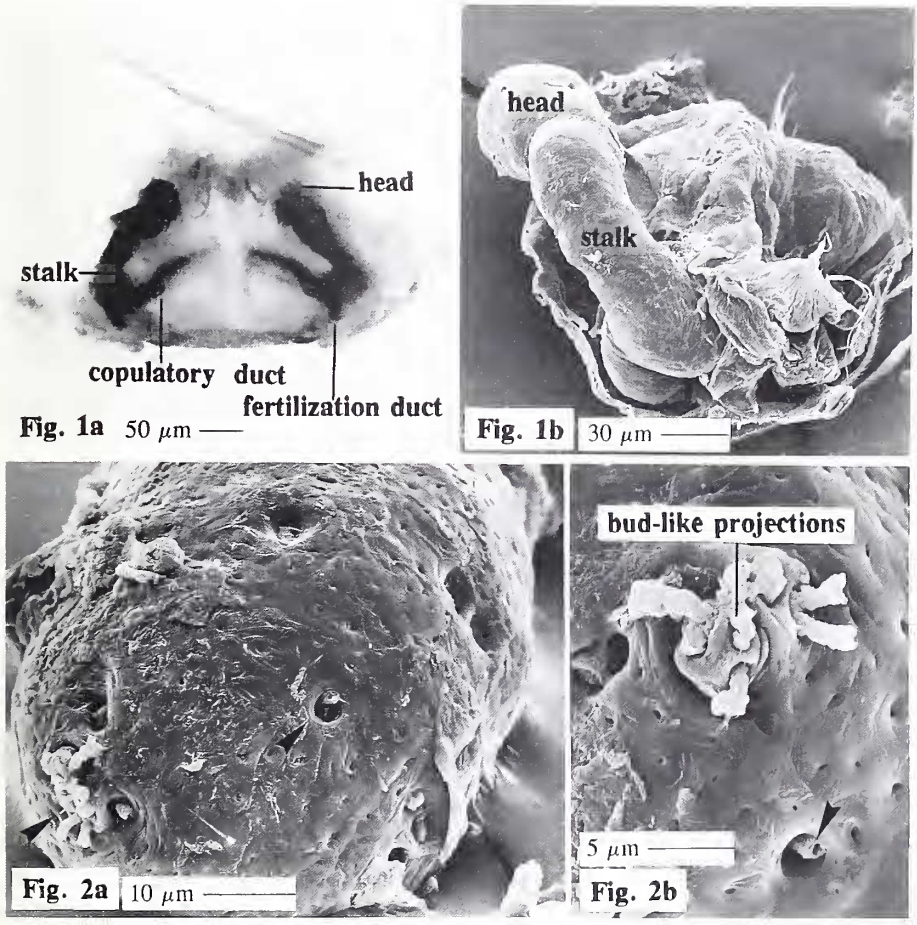
The female genitalia of *Pardosa lugubris* are of the entelegyne type. In this spider species each spermatheca is elongated and consists of a stalk region and a blind ending head of the spermatheca which is slightly dilated. The copulatory opening leads via the short, thick-walled copulatory duct into the stalk region of the spermatheca, from where the short fertilization duct arises to the posterior end of the epigynal fold leading into the uterus externus (Figs. 1a, 1b).

The cleared epithelial side of the spermathecal cuticle exhibits approximately 20 more or less evenly distributed complex pores (primary pores), restricted to the head of the spermatheca. In *P. lugubris* most of the primary pores are cavities penetrated by 1–2 cuticular ductules, whereas few of the primary pores form bud-like projections with 3 or more ductules (Figs. 2a, 2b).

Our ultrastructural studies on the spermathecal epithelium shows two ectodermal glands separated from the haemolymph by a basement membrane. One gland consists of type III gland units, whereas the other gland consists of type I gland cells according to the classification of NOIROT & QUENNEDY (1974, 1991).

Each type III gland unit is composed of several cell types: The prominent secretory cells exhibit a basal labyrinth. The cytoplasm contains mitochondria, well developed Golgi regions and rough endoplasmic reticulum filled with large amounts of some fibrous material (Fig. 3). The microvilli of several secretory cells (Fig. 4) project into a common lumen which terminates in a cuticular ductule leading to a primary pore.

Once we observed two ciliary structures of the 9X2+0 axonemal pattern within the proximal part of the ductule (Fig. 5). The ductule is surrounded by a canal cell. The secretory cell as well as the canal cell are surrounded by several narrow sheath

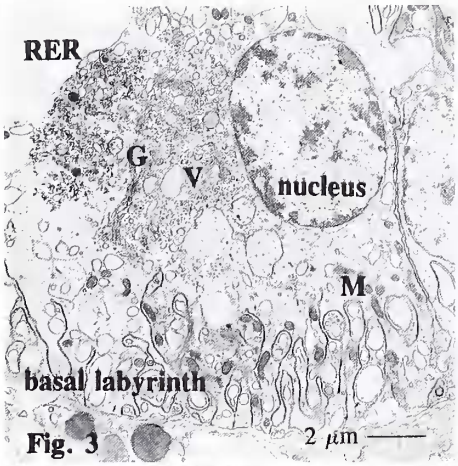


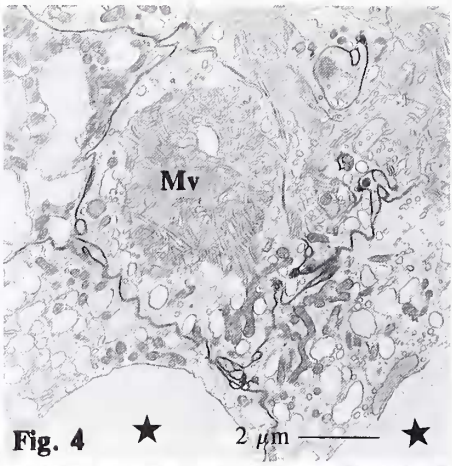
FIGS 1–2

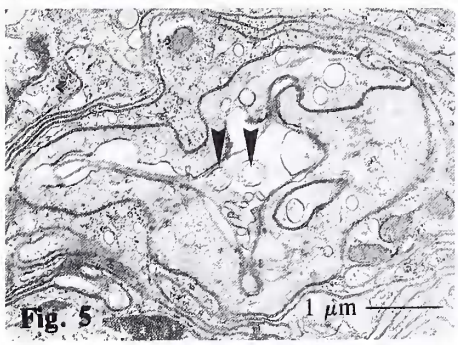
Fig. 1a. Dorsal view of the vulva. The copulatory and fertilization duct lead into the stalk region of the spermatheca, which leads into the slightly dilated head of the spermatheca. Fig. 1b. SEM on the spermathecal stalk and the head of the spermatheca. Fig. 2a. Overview of the cleared epithelial side of the head of the spermatheca. (Pores indicated). Fig. 2b. Higher magnification. Primary pores as "simple" cavities with cuticular ductules (arrow) or bud-like projections with several ductules.

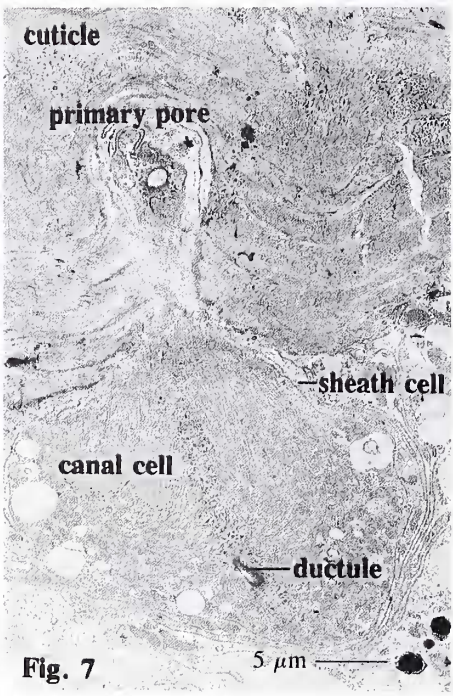
cells. Their cytoplasm is electron lucid and only few organelles were observed. In contrast to the secretory cells, the nuclei of the sheath cells are not located basally, but more apically near the cuticular ductule. The canal and sheath cells convey the cuticular ductule into the primary pore (Figs. 6, 7).

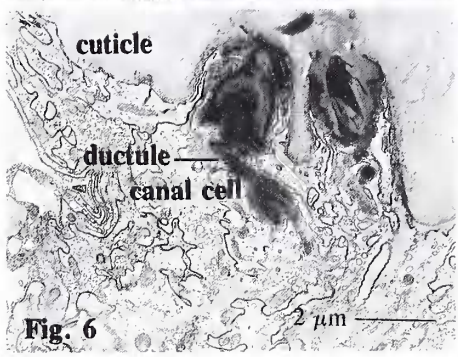
Furthermore, a single secondary pore is located on the spermathecal stalk which is not detectable by the scanning electron microscope, but by light and











FIGS 3–7

Fig. 3. A secretory cell showing a basal labyrinth. The cytoplasm contains rough endoplasmic reticulum (RER), filled with some fibrous material, mitochondria (M) and well developed Golgi region (G). V Golgi vesicles. Fig. 4. Several secretory cells which project microvilli (Mv) into a common lumen. Prominent RER cisternae are filled with fibrous material (asterisk). Fig. 5. Proximal part of the ductule surrounded by a canal cell. Ciliary structures of the 9X2+0 axonemal pattern within the lumen (arrows). Fig. 6. Canal cell conveying the cuticular ductuleinto the primary pore. Fig. 7. Canal and sheath cells leading into the primary pore.

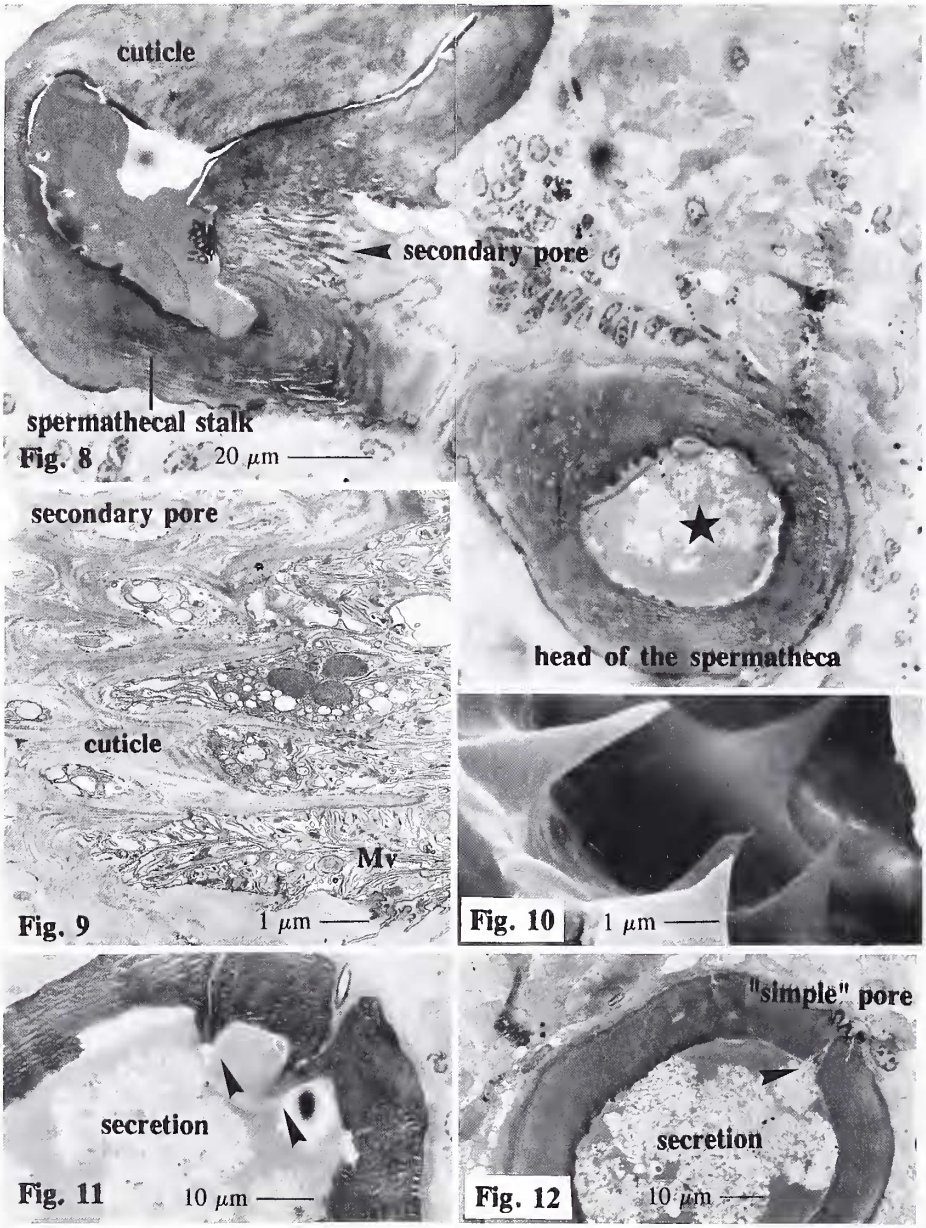
transmission microscopy as it is hidden by the copulatory duct. The secondary pore is plain and therefore does not extend into the lumen of the stalk (Fig. 8). The cell processes of the second gland already mentioned lead to this porous plate. The gland is built up by a single cell type: The nuclei are located basally and are of elliptic shape. In this region, we found also mitochondria, rough endoplasmic reticulum and Golgi regions. Apically the cells narrow and their microvilli penetrate the perforated cuticle of the spermathecal stalk (Fig. 9). The spermathecal epithelium is only sparsely innervated.

The luminal cuticular spermathecal surface shows distinct projections, too. The basal part of the stalk region exhibits many solid thornlike projections (Fig. 10). In contrast, in the head of the spermatheca some few primary pores end within cannular, hollow cuticle projections (Fig. 11), whereas most other primary pores are "simple" pores through which exudation into the lumen occurs (Fig. 12).

DISCUSSION

Perforations similar to primary pores have been described in many spider species. They occur in haplogynes (Cook 1966; Brignoli 1976; Coyle *et al.* 1983) as well as in entelegynes (Strand 1906; Sierwald 1989; Bennett 1992). Observations of the fine structure of primary pores of *Segestria senoculata* (Linné, 1758) (Segestriidae) (unpubl.), *Antrodiaetus unicolor* (Hentz, 1842) (Antrodiaetidae) (unpubl.), *Pholcus phalangioides* Fuesslin, 1775 (Pholcidae) (Uhl 1994) and *Amaurobius fenestralis* (Stroem, 1768) (Amaurobiidae) (Suhm & Alberti 1993) have shown a structure similar to that of *Pardosa*. This study provides further support for the hypothesis of primitive presence of primary pores in the vulva of all spiders as suggested by Sierwald (1989). The occurrence of further, complex pores restricted to the stalk region has only been reported in few spider species, which are all of the entelegyne type. In the spermatheca of *Agelena labyrinthica* (Clerck, 1757) (Agelenidae), Strand (1906) described a single perforation resembling that of *A. fenestralis* recorded by Suhm & Alberti (1993). There is also an obvious similarity in the fine structure of the secondary pore of *A. fenestralis* located within a concavity and the porous plate of *Pardosa lugubris*, because both are located in the stalk region and show a similar fine structure. Therefore, these structures might be homologous. Bennett (1992) described single pores in the stalk region as synapomorphic for dictynoid spiders (referring to Amaurobioidea, Dictynoidea sensu Platnick 1989), hence he termed them dictynoid pores. Although Bennett supposed no "dictynoid" pores for lycosids and *Amaurobius*, we found pores in the stalk region in both of them. As most studies on the female genitalia are SEM studies, the porous plate might not have been detected, since it is possibly hidden by the copulatory or fertilization duct as it is in *P. lugubris*. Therefore, this pore may be more generally distributed than suggested by Bennett. In our opinion the secondary pore needs to be reevaluated as a taxononic character state for dictynoid spiders.

Primary pores have been regarded as glandular ductules. However, as we already described in *A. fenestralis* (Suhm & Alberti 1993) we also found some



FIGS 8–12

Fig. 8. LM study on the spermatheca shows the porous plate (secondary pore; arrow). The head of the spermatheca is filled with some material (asterisk). Fig. 9. The narrow apical cell regions of the secondary pore gland show microvilli (Mv) leading to the pore. Fig. 10. SEM showing solid thornlike projections in the lumen of the basal part of the stalk region. Fig. 11. Cannular cuticle projections (arrows) protruding into the lumen of the head of the spermatheca. Fig. 12. "Simple" pores in the cuticle of the head of the spermatheca through which exudation of some material appears (arrow).

similarities to typical arthropod sensilla like: ciliary structures (receptor cells?), several auxiliary cells (canal and sheath cells) and a cuticular ductule that may correspond to the dendritic sheath.

In the case of *P. lugubris* ciliary structures within the glandular duct were found only once. Furthermore, the epithelium does not seem to be strongly innervated. Therefore, it might be possible that most of the primary pores function as ectodermal glands, whereas only few other pores may have another/additional function. The cannular projections found in the lumen of the head of the spermatheca may allow a better mixing of the secretion with the sperm mass, whereas the solid thornlike structures observed in the stalk region might retain the sperm mass within the spermatheca as Osterloh (1922) suggested in *Pardosa amentata* (Clerck, 1757). The glandular cells leading to the secondary pore seem to exude some product into the spermathecal lumen, too. The function of the two ectodermal glands found in *Pardosa* remains unclear, but their secretion might contribute directly to sperm nutrition, activation and/or move the sperm from the receptaculum into the fertilization duct prior to oviposition as already suggested by many other authors. Unfortunately none of the substances were isolated for chemical or biological characterization, and without additional information, their precise contribution to reproduction must remain unsolved.

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